Pre-Synopsis for Ph.D.

Entitled

Molecular Characterization of Secondary Metabolites from Medicinal Plants against Pathogenic Bacterial Diseases in Animals

Submitted to

Swami Ramanand Teerth Marathwada University Nanded-431606, Maharashtra State, India

(UGC recognized NAAC Re-accredited 'B^{++}' grade state university)

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DECEMBER 2024

Title of Ph.D. Work

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1. Introduction:

India has a rich and diversified flora. It is seen that synthetic drugs could pose serious problems, are toxic and costly. In contrast to this, herbal medicines are relatively non toxic, cheaper and are eco-friendly. Moreover, the people have used them for generations. They have also been used in day-to-day problems of healthcare in animals. 25% of the drugs prescribed worldwide come from plants.

The rising number of people is turning to herbal medications since they are thought to have negligible or no negative effects. 80% of people in Asian and African nations rely on traditional medicine for their basic medical requirements. Traditional herbal treatments are the most profitable type, bringing in billions of dollars annually. Since 40% of the plants include essential components that can be used to make prescription pharmaceuticals, researchers look to traditional remedies as a guidance.(1)According to reports, the global market for herbal products was worth $80 billion in 2011–2012. The Indian herbal products market is about Traditional medicine (herbal medicine) is defined by the World Health Organization (WHO) as "the total of the knowledge, skills, and practices based on the theories, beliefs, and experiences from other cultures, whether or not they can be explained, that are used to maintain health as well as help prevent, diagnose, treat, or improve physical and mental illness.(2) About 25% of current medications are known to have originated from plants that were originally used traditionally. Three out of four patients with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) in Africa, North America, and Europe utilize some type of conventional treatment for a variety of symptoms and problems.

Herbal remedies used in veterinary medicine include plant-based medications used for therapeutic, preventative, or diagnostic purposes in animal healthcare. The use of herbal medicines for both human and animal health care has a long history that dates back thousands of centuries.

In India's rural areas, veterinary medications cover the smallholder knowledge, abilities, procedures, practices, and beliefs around the care of their livestock. Due to financial constraints, these smallholder farmers are unable to invest in the high-quality care of their animals; in contrast, high-end medical treatment is typically provided by costly but powerful synthetic medications. The consequences of synthetic pharmaceuticals, such as harmful compounds that stay in meat and antibiotic residues that cause antibiotic resistance in people and most of useful domestic animals,beside resistance development certain antibiotics trigger various side effects of commercially available animal drugs.[4]

The components in many herbs (whole plants) have many actions that are combined to make a single medicine. In the interest of animal health, it would be prudent to consider the risk-benefit ratio based on the available scientific information and the prescriber's experience.[5] However, various ethnomedicinal plants used in Indian healthcare systems have been investigated scientifically for nearly three decades [6]. Since then, the Government of India has made several attempts to investigate the possibility of evaluating these systems for their therapeutic potential as they were originally practiced, as well as to generate data to include them in national healthcare programs in utilizing their potential from various healthcare perspectives [7]. According to the WHO report (2018), 66% of India’s population resides in rural areas with a large dependency on agriculture. In the Himalayan region, the rearing of animals to supplement the family income and sustain crop production constitutes an important component of the rural economy of the region [8,9,10]. In the mixed crop–livestock farming systems, livestock and food production systems are closely integrated in the Himalaya [11]. The Himalayan region’s rural economy is based mostly on animal husbandry, and the growth of this industry could raise the standard of living in rural areas. Farmers look after their livestock by using ethno-veterinary practices [12]. To keep domestic animals healthy and productive, ethno-veterinary remedies are widely and very successfully utilized for basic healthcare. People in remote rural areas continue to rely heavily on herbal and common domestic remedies to treat veterinary ailments. The system comprises conventional beliefs, knowledge, expertise, approaches, practices and traditions of a particular society [13,14].Medicinal herb for blood disorder and low immunity are taking much attention.

The immune system is simultaneously the most challenging and self-evident to address of all the systems covered in this section. Practitioners are commonly asked how to "stimulate the immune system," but scientists and herbalists agree that due to the intricacy of immune function, such an approach is at the very least naive and at the very best, not advised. Immune function is an ecological balance that the body achieves with the help of endocrine and neurologic processes.

Above citations clears that there must be use of ecofriendly alternatives to relive the load of chemical allergy and resistance development of certain animal drugs. keeping these views in to the mind present investigation have spotlight upon bioactive elements of various drug molecules isolated from medicinal plants.

1.1 Study site:

The district Latur (18.4088° N, 76.5604° E) this district has an historical background. The King 'Amoghvarsha' of Rashtrakutas developed the Latur city, originally the native place of the Rashtrakutas. The Rashtrakutas who succeeded the Chalukyas of Badami in 753 A.D called themselves the residents of Lattalut. The Latur geographical location shows that it situated south-eastern part of the Maharashtra state. At Latur we will choose different sample sites to collect medicinal plants with potential bioactive elements some prominent sample sites are

1.1.1The Sanjivani Bet

This also called as Wadval Nagnath Bet is very famous among tourists and scientists because of its biodiversity and producing a very rare species of medicinal plants. The rare plants grow in the month July to September (Uttara Nakshatra).

1.1.2Hattibet-Deverjan

This is another hotspot for medicinal plants in Latur district at block Udgirsouth-eastern part of the Maharashtra state. On a small hill is the Tomb of Gangaram Maharaj. Around it many rare medicinal plants are present.

2. Review of Literature

2.1 International Status

Infectious diseases account for approximately one-half of all deaths in tropical countries.(15)Medicinal plants have been traditionally used for different kinds of ailments including infectious diseases.(16) Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. Historically, plants have provided a good source of anti-infective agents. The isoquinoline alkaloid, emetine, obtained from the underground part of Cephaelis ipecuanha, and related species, have been used for many years as an amoebicidal drug for the treatment of abscesses due to the spread of Escherichia histolytica infections. Quinine, an alkaloid that occurs naturally in the bark of the Cinchona tree, is another important drug of plant origin with a long history of usage against malaria. The higher plants have made important contributions in areas beyond anti-infective, such as cancer therapies. Scientists from divergent fields are investigating plants with an intention to discover valuable phytochemicals. Laboratories all over the world have found literally thousands of phytochemicals which have inhibitory effects on all types of microorganisms in vitro.(17) A traditionally used tuber found growing along the banks of the Zambezi River and used commonly throughout southern Africa has become a popular traditional treatment for HIV-related illnesses. It is widely called the 'African potato', but the botanical name is *Hypoxis hemerocallidea* (formerly *H. rooperii*) and it has been traditionally used as food and medicine. The tuber is reported to help maintain or increase CD4-cells and boost cellular immunity in the body. Traditional health practitioners in southern Africa use it for managing HIV infections, cancer, TB, influenza, arthritis, psoriasis and common cold(18)

2.2National Status

Ethnobotany is completely in effectively latest grassland of investigation, if in this grassland plants consider thoroughly and systematically it will permit result of broad value of the archeologists, anthropologist, plant geographer, ethnobotanist, linguistics botanist and Phyto chemists.(19) described ethnomedicinal plant product apply by the regional

normal practitioners in Mount Abu, Rajasthan. They explained 19 plant species apply by the normal practitioners of this area.

Fitness and medicinal effects of lemon has been described by Mohanapriya (2013). Lime fluid is applied for treatment of scurvy, skin, care, constipation, peptic, ulcer, respiratory, disarray, gout, piles, urinary disarray and pressure erosion. Medicinal effects of Murraya koenigii has been considered by Kumar et al.(20) It can be exerted as medicines to antidote different ailments. It can be applied as anthelmintic and also acts as febrifuge sustenance purifier sedative, anti-inflammatory, figure pain, kidney pain and vomiting. Herbaceous healing and medicinal plants of region Samba of Jammu province, Jammu and Kashmir was described.(21)

2.3 Regional Status and Scope of Study

No attempt had been recorded from Latur regards Molecular Characterization of Secondary Metabolites from Medicinal Plants particularly at Sanjivani and Hatti Bet. Most attempts show extract which containing many more secondary metabolites but never shows single bioactive compound and their effect on particular disease-causing bacteria in animals.

3. Objectives of Research

- Identification of efficient medicinal plants containing antibacterial properties.
- To isolate secondary metabolites from medicinal plants.
- To study molecular characterization of selected secondary metabolites, present in medicinal plants.
- To study antimicrobial effect of these secondary metabolites on different disease-causing bacterial strains.

4. Materials and Methods:

4.1 Plant Collection and Identification

Different plants will be selected that containing medicinal properties. These plants will be selected for the extraction of secondary metabolites and to check their antibacterial effects on different disease-causing bacteria.

4.2 Extraction and Isolation

Chosen plants from the sample sites will be collected and their fresh leaves will be washed in clean water. After that theses leaves will be dried and grinded into fine powder. Then this mixture will filter through a clean muslin cloth. Followed by filtration through Whatman no.1. This extract will dry in a rotary evaporator at 37ºC. The dried extract was stored in a refrigerator for further use. Secondary metabolites will be extracted using solvents (ethanol, methanol, etc.) via maceration or Soxhlet extraction. Isolation will be achieved through chromatographic techniques like TLC, column chromatography, and HPLC.

4.3 Molecular Characterization

The molecular characterization will be achieved by using different structural elucidation techniques like HPLC for quantification, GC-MS for identifying volatile components, NMR (Nuclear Magnetic Resonance), etc.

4.4 Antibacterial Assay

Sets of the experiment will be conducted by using selected animal pathogens. For each bacterium different secondary metabolites will be tested to check its anti-bacterial property by using cross-streak method.(22) The control petri dishes will maintain only pathogenic bacteria in animals and without the addition of secondary metabolites. Starting from the first day, after the addition of secondary metabolites the number and growth of bacteria will be monitor using Zone of inhibition tests. Zone of inhibition tests will assess the potency of the extracts against the pathogenic bacteria causing diseases to animals.

5. Scope of study: Present research work will useful for

- Identification of potential medicinal plants with significant antibacterial activity.
- Isolation and molecular characterization of bioactive secondary metabolites.
- Development of a database of effective compounds targeting specific bacterial pathogens in animals.

6. Year wise plan of work and targets to be achieved

First Year:

- Collection of review of literature.
- Collection of medicinal plants source

Second Year:

- Isolation of secondary metabolites and Selection of Medicinal Plants containing Potential Bioactive Compounds
- Study of molecular characterization of isolated secondary metabolites

Third Year:

- Study of antimicrobial potential of isolated secondary metabolites on various animal pathogenic bacteria
- Thesis writing, typing, binding, Pre-Ph.D. Viva and Thesis submission.

7. Proposed work plan with Tentative Chapter Scheme

- **Chapter I**: Introduction
- **Chapter II**: Review of literature.
- **Chapter III**: Materials and Methods
- **Chapter IV:** Results and Discussion
- **Chapter V:** Conclusion
- **Chapter VI:** References

Future perspective:

- Identification of new Secondary Metabolites from Medicinal Plants against Pathogenic Bacterial Diseases in Animals
- Making Database for Future Researchers

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